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Review

Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning

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Abstract

In order to study mechanisms involved in the etiology of human affective disorders, there is an abundant use of various animal models. Next to genetic factors that predispose for psychopathologies, environmental stress is playing an important role in the etiology of these mental diseases. Since the majority of stress stimuli in humans that lead to psychopathology are of social nature, the study of consequences of social stress in experimental animal models is very valuable. The present review focuses on one of these models that uses the resident-intruder paradigm. In particular the long-lasting effects of social defeat in rats will be evaluated. Data from our laboratory on the consequences of social defeat on emotional behavior, stress responsivity and serotonergic functionality are presented. Furthermore, we will go into detail on hippocampal functioning in socially stressed rats. Very recent results show that there is a differential effect of a brief double social defeat and repetitive social defeat stress on dendritic remodeling in hippocampal CA3 neurons and that this has repercussions on hippocampal LTP and LTD. Both the structural and electrophysiological changes of principal neurons in the hippocampal formation after defeat are discussed as to their relationship with the maintenance in cognitive performance that was observed in socially stressed rats. The results are indicative of a large dynamic range in the adaptive plasticity of the brain, allowing the animals to adapt behaviorally to the previously occurred stressful situation with the progression of time.

Keywords: A; B; C

Contents

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1. Introduction

Stressful life events are generally considered as having precipitating effects on the development of human psychopathologies such as anxiety and clinical depression [1–3]. In order to study mechanisms involved in the etiology of these affective disorders, there is an abundant use of various animal models. This large variety in models used reflects Selye's concept of a core physiological response pattern to homeostatic challenges [4]. The effectiveness of stressors applied in these models to induce a pathology-like state that resembles a failing adaptive capacity, is dependent on the absence of the animal's possibilities to cope with the challenge [5]. Although large individual differences exist in the way animals as well as humans cope with stress [6], these coping strategies in general encompass adequate behavioral, physiological and neurobiological tools to diminish the impact of the stressor. Since the aim of many animal stress models is to mimic human stress-related psychopathologies, researchers aim at inducing a state in the experimental animals that bears an obvious resemblance to the behavioral or physiological signs of these clinical disorders. In order to reach this state, frequently stressors are applied with a relatively high intensity that have a chronic character. Usually, the behavioral, physiological and neurobiological consequences are studied during or shortly after the end of the stress period. Only few studies focused on longer-lasting, persisting effects of this prolonged stress exposure. There appears to be, however, a growing interest in the study of long-term behavioral and physiological consequences of short-lasting, episodic-like stressors, which will be the focus of this review.

2. Long-lasting effects of short-lasting non-social stress episodes

Various papers have shown that a short-lasting stress-exposure can induce long-lasting changes in experimental animals. Exposing rodents to a predator like a cat induces an increased anxiety that lasts for several weeks as observed in exploratory behavior in the elevated plus maze [7]. It was also shown that single administration of the anxiogenic β-carboline, FG-7142 elicits behavioral changes that last for at least 6 weeks [8]. A number of short-lasting (2 h) daily sessions of inescapable stress inhibits spontaneous wheel running behavior also for 6 weeks [9]. Several other publications indicate long-lasting behavioral [10,11] and neuroendocrine [12–15] effects of a single exposure to non-social aversive stimuli like foot shocks and restraint. These studies further indicate that part of these effects are progressive and consolidate with the passage of time. Various single stressors like foot shocks and challenges with lipopolysaccharide (LPS) and IL-1β can provoke a shift in the balance between vasopressin (AVP) and corticotrophin-releasing factor (CRF) content in the external zone of the median eminence in the direction of AVP [16–18] or in the expression of AVP in CRF hypophysiotrophic neurons in the paraventricular nucleus of the hypothalamus [19]. This may cause an enhancement in hypothalamic-pituitary-adrenocortical (HPA) axis functioning [20]. Also steroid signaling of the brain is affected for longer time periods in stressed animals. Exposing rats to a single, 2.5 h lasting, prolonged stress paradigm (SPS) results in a severe decrease in the ratio between hippocampal mineralocorticoid and glucocorticoid receptors (MR/GR) that lasts for at least two weeks [21]. Another example of lasting effects of brief challenges can be observed in drug-sensitization. Single exposure to neuroleptics [22] or amphetamine [23] causes a context-independent sensitization to a re-exposure to the drug, which intensified over time.

3. Long-lasting effects of social defeat exposure

All these stressors mentioned above being capable of inducing long-lasting behavioral, neuroendocrine and neurobiological effects are of non-social nature. However, the majority of stress stimuli in humans that lead to psychopathology are of social nature [24]. Since many studies have indicated that different types of stress can elicit qualitatively different patterns of behavioral and physiological stress responses [4], the research on the consequences of social stress in experimental animal models, therefore, is crucial. For this reason there has been extensive research on mechanisms involved in stress-related disorders in animal models applying social stress. The most frequently used models for rodents are the social defeat paradigm and the social colony model. In the first, experimental male animals are introduced into the territory of an aggressive male

conspecific. The intruder is rapidly investigated, attacked and defeated by the resident. To ensure the desired outcome of the social conflict, residents usually have a higher bodyweight and are familiarized with fighting. They usually belong to a strain with relatively high levels of aggression [25–33]. In a social colony, male and female rats are living together in semi-natural conditions. After the development of a social structure dominant and subordinate males are identified. There are major behavioral, neuroendocrine, physiological and neurobiological differences between individuals of these two hierarchy levels [34,35].

In this review, we will restrict ourselves to describing the long-lasting effects of social stress in the social defeat paradigm where male rodents are exposed to, or threatened to be exposed to aggressive male conspecifics. The large amount of data obtained in this model show that stress of social defeat produces intense acute and long-lasting behavioral and physiological responses that are accompanied by substantial changes in brain neurochemistry (for reviews see [4,25,36]. Koolhaas et al. [25] indicated that the temporal dynamics of these responses is differential, depending on the parameter of study. These reviews [4,25,36] are very elaborate in describing effects of social stress on social and non-social behavior and on neuroendocrine, physiological and central nervous functioning. We will, therefore, not repeat all the information that was made available in these publications. Instead we will cluster some of the changes following defeat in a temporal dynamic perspective, particularly focusing on long-lasting effects.

In general it can be concluded that the effects of social defeat on baseline activity of cardiovascular, endocrine and autonomic nervous systems appear to be relatively short-lasting, i.e. not lasting much longer than 24–48 h after defeat [32,37–40]. Some effects, like the reduction of circadian amplitudes of home cage activity, body temperature and heart rate, can last for up to one week after the last defeat [29,30,33,41–45]. An interesting finding is that this period can be extended to several weeks by defeating rats that previously have had 10 subsequent daily 'winning' experiences [46]. The authors reported a large individual variation in the duration of the consequences of a social defeat that correlated negatively with the amount of counterattacks during the conflict. In this respect also the housing conditions following defeat play a major role in the magnitude and duration of the social stress effects. Long-lasting effects are only observed in single housed rats and not in socially housed animals [47–49]. The changes in HPA-axis regulation can also be long-lasting as we previously showed in the impairment of the glucocorticoid feedback inhibition of activation of this axis that lasted for several weeks [47,50].

True long-lasting effects (lasting for several weeks or even months) are reported on behavioral responses to social and non-social challenges of various nature [29,47,51]. A number of publications indicated that the behavioral anticipation to a hedonic stimulus like sucrose consumption can even be impaired for a period longer than 100 days after defeat [48,52,53]. Since a diminished interest or pleasure is one of the DSM-IV criteria for clinical depression, the authors concluded from these results that a brief social defeat experience can induce long-lasting behavioral signs of depression.

As mentioned above, animal stress models are applied in order to gain our understanding of mechanisms involved in human stress-related psychopathologies like anxiety, depression and post-traumatic stress disorders (PTSD). Although it is generally acknowledged that it is practically impossible to distinguish these behaviorally complex and heavily intertwined clinical disorders in animal models, nevertheless many attempts have been made to define the severe and lasting effects in the stress models into terms of these human diseases. Behavioral changes, like a decreased social interaction [29,48] and anhedonia [48], next to physiological [30], neuroendocrine [35,50,54], and neurobiological [50,55–58] consequences of social stress are interpreted as signs mimicking certain aspects of human depression. Behavioral and pharmacological tools in treating human depression were also applied in socially stressed animals and many of these treatments proved to be beneficial in reducing the behavioral, physiological, neuroendocrine and neurobiological changes following defeat. Sleep deprivation [27,59], antidepressant drugs like clomipramine [27,54,60], imipramine [52,53] and fluoxetine [31] as well as social interaction [47–49] prevented many of the consequences of social stress. For this reason the social stress model is generally interpreted as modeling human affective disorders like stress-related depression.

Next to presenting some unpublished data from our laboratory on the consequences of social defeat on behavior in the elevated plus maze, on sensitization to subsequent mild stressors and on the desensitization of serotonergic 5-HT_{1A} receptors, we will focus on hippocampal functioning in socially stressed rats. This, because it is targeted intensively by stress hormones like glucocorticoids. Recently published and unpublished findings will be presented, supporting the view that social defeat stress induces major structural changes in subregions of the hippocampal formation. Furthermore we will try to relate these structural changes to electrophysiological properties and functional behavioral consequences of social stress involving this brain structure.

4. Contextual and generalized anxiety provoked by previous social defeat

Effects of previous stress experience on fear and anxiety in general have been studied acutely and at very short intervals after the stress exposure [61–63]. Korte and de Boer [63] characterized fear as a behavior caused by real and immediate dangers while behavioral anxiety is caused by unreal or imagined threats. Using this terminology, fear can be elicited by placing animals in a dangerous situation like the home cage of an aggressive male conspecific. This

can be performed acutely with the resident attacking the intruder. One can, however, also study long-term effects of a previous defeat on conditioned fear behavior that is elicited in the context of the previous stress exposure, i.e. in the resident's cage. The resident does not necessarily have to be present to elicit this kind of conditioned fear in the experimental rats. To study the long-term effects of a previous defeat on conditioned fear we analyzed behavior of intruders before and 35 days after defeat in the resident's home cage. On these two days, resident male rats were placed in small wire mesh cages on the left side of the resident cage. The home cage of the intruder was placed in the right half of the resident cage. After 3 min the intruders were allowed to explore the resident cage freely for 10 min. Fig. 1A shows that 35 days after a double defeat the behavior in the rats' own home cage, being placed into the resident's cage, is completely different from the behavior in that situation when tested prior to the defeat. Non-defeated controls hardly change their behavioral activity the second time they are exposed to this environment. Defeated rats groom and rear less whereas immobility or freezing behavior is increased significantly. In Fig. 1B the Perspex top of the home cage is removed and animals can freely explore the confined resident in the wire mesh cage and the resident cage. Defeated rats freeze more and spend more time exploring their own home cage bottom. They clearly avoid the resident (rats spend 39% less time exploring the left side of the cage, where the resident is placed), which explains the decreased exploration of resident's wire mesh cage. The major behavioral changes that occur 35 days after the defeats indicate that animals remember the context of their aversive experience. The high freezing scores after defeat allow the assumption that defeat elicits a clear contextual conditioned fear response 35 days later. This experiment also indicates that social defeat does not impair contextual memory performance. Since hippocampal functioning plays an important role in contextual memory [64–66], these results indicate that in this behavioral paradigm there are no signs of long-lasting impaired hippocampal functioning following defeat. However, one could state that this behavioral test is not a highly refined tool making it not possible to differentiate between subtle changes in hippocampal functioning.

Emotional behavior can also be studied in situations that are not directly related to the context of the previous stress experience. This approach in studying emotion in experimental animals provides information on general anxiety levels instead of fear. Exploration of the so-called elevated plus-maze has been widely used as a behavioral tool to acquire this information [62,63,67–71]. A reduced exploration of the open arms of the maze has been interpreted as reflecting increased anxiety levels in experimental animals. Anxiolytic compounds increase the amount of time spent on exploration of the open arms, whereas anxiogenics do the reverse [67,68,70,72–75]. Heinrichs et al. [74,75] showed that shortly (5 min) after social defeat rats were more anxious on the elevated plus-maze. Korte and de Boer [63] showed that in rats, social defeat produces a significantly increased anxiety in the plus-maze that lasted 1 day. No statistical differences between stressed and unstressed groups were found on 1, 2 and 3 weeks after defeat. Ruis et al. [47] reported that after a single defeat exposure, individually housed rats, of a wild-type strain, showed an increased anxiety that lasted up to 14 days after the defeat.

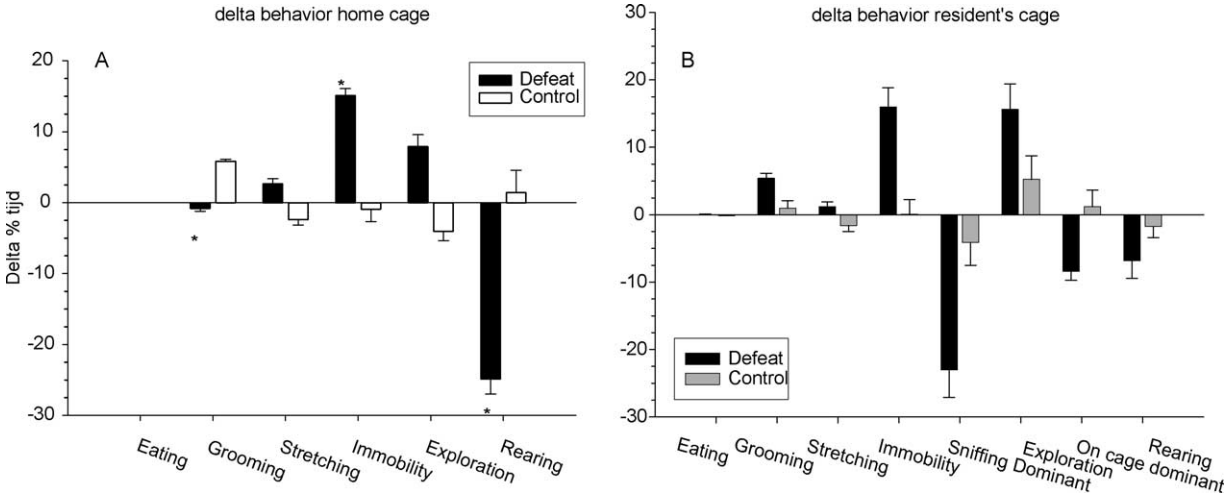

Fig. 1. The left graph (A) shows the behavioral change in defeated ($n=9$) and non-defeated ($n=9$) rats in their home cage when it is placed for 3 min into the right side of the resident's cage 35 days after a defeat experience in that cage. Grooming and rearing was decreased as compared to non-defeated rats, whereas immobility was higher in defeated rats ($p<0.01$). The resident male was placed in a wire mesh cage, which was placed on the left side of the resident's cage. Behavioral change is plotted against the behavior in the same test situation 3 days before defeat. The right graph (B) shows behavior of the same rats when the top of the home cage was removed and rats were allowed to freely explore the resident's cage for 10 min. Immobility and exploration (mainly the floor of the 'intruder's' home cage) was increased ($p<0.01$ and $p<0.05$), while exploration of the resident ($p<0.01$), sitting on top of the cage of the resident ($p<0.05$) and rearing ($p<0.05$) was decreased.

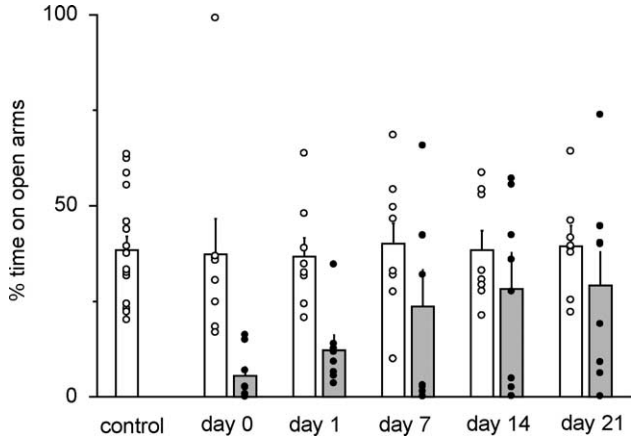

Fig. 2. Percentage of time spent on exploring the open arms of the elevated plus maze before and 5 min (day 0), 24 h (day 1), 7, 14, and 21 days after a single social defeat. Control group on the far left side of the bar graph consists of 16 animals. Each other bar (white for non-defeated and gray for defeated rats) represents a group of eight rats that all have been exposed only once to the maze. Significant group differences were observed at day 0 and 1 ($p < 0.05$).

Housing conditions apparently play a major role in these effects since housing defeated rats in social groups prevented this effect completely [47]. Albonetti et al. [76], however, found no stress effect on behavior in the plus maze 1 day after defeat. Fig. 2 shows the data of Korte and de Boer [63] again but now presented as individual scores of animals in the plot. As can be observed in the individual scatter plot in the bars, large individual differences existed up to 3 weeks after social defeat. Almost half of the animals did not recover behaviorally from the defeat stress. Although the mechanism behind this variation is not known, it might well be that these findings are related to the subjective experience of the experimental animal during the physical conflict. This idea is supported by the finding that the level of perceived control over the stressor is crucial for the outcome of defeat on anxiety behavior in the plus-maze [63]. Animals that can actively control their stress do not show anxiety in the maze. Also Meerlo et al. [46] presented data indicating that animals that fight back during the conflict, even though they are defeated in the end, show less severe behavioral and physiological changes following stress. Therefore, taking notice of the large variability in long-term anxiety following defeat, it is possible that there is a differentially perceived controllability in defeated rats.

5. Progressive development in hypersensitivity to mild non-social stress after previous social defeat

Post suggested [2,77] that stress induces a cascade of neurobiological processes that leads to an increased vulnerability to subsequent stressors that ultimately may result in stress-related mood disorders. There are a number of papers that show that social-defeat stress, unlike many other environmental stressors, does not result in habituation [33,78] nor in sensitization [79,80] upon repeated presentation. Actually the issue of sensitization following stress is most frequently raised in studies on the increased vulnerability to drugs of abuse. Behavioral sensitization is defined as an enhanced response to a challenge dose of a stimulant drug, like amphetamine and cocaine, after a period of intermittent administration of this drug [23]. An increased response to stimulant drugs also has been described in animals after having been exposed to various stressors, and this increased stimulant response usually is referred to as 'cross-sensitization' [81]. In particular Miczek et al. have investigated the occurrence of cross-sensitization after social defeat stress [78,82–85]. From these results it was concluded that social stress induces changes especially in dopaminergic activity in the brain, which renders the individual more vulnerable to acquiring psychomotor stimulant self administration [78].

Although reports do exist [27,86,87], the occurrence of heterotypic stress-sensitization has not received a lot of attention in social stress research. If experimental animals are equipped with intraperitoneally implanted biotelemetry minitransmitters, one of the weekly recurrent stressors for laboratory animals could easily be used for this purpose; the cleaning of bedding of the home cage. This repeated cage-cleaning procedure offers via biotelemetry an opportunity to study whether animals that have been defeated are more sensitive to subsequent exposures to different types of stress. In Fig. 3 the increase in body temperature, heart rate and locomotor activity following the presentation of a clean home cage to rats is shown 1 week before, and 7 and 21 days after a social defeat. It is clear that there is a progressively increasing physiological and behavioral response to this relatively mild stressor. One week after the defeat the rise in temperature and heart rate is not yet significantly different from the response before defeat, but 2 weeks later there is a clear and significant increase in this response in the same animals. This increasing physiological response with time to clean bedding goes together with an increased locomotor activity response. Three weeks after defeat rats the locomotor response to this mild stressor is more than double as compared to pre-defeat activation. These results clearly show that after social defeat gradually a sensitization develops to heterotypic stressors, indicating that central nervous systems involved in handling stress responses become progressively more sensitive to challenges with the passage of time after social defeat.

6. Desensitization of serotonergic 5-HT$_{1A}$ receptors following social defeat

Opposite to the sensitization to heterotypic stressors as indicated in the section above, the sensitivity of receptors of neurotransmitter systems that become activated after defeat

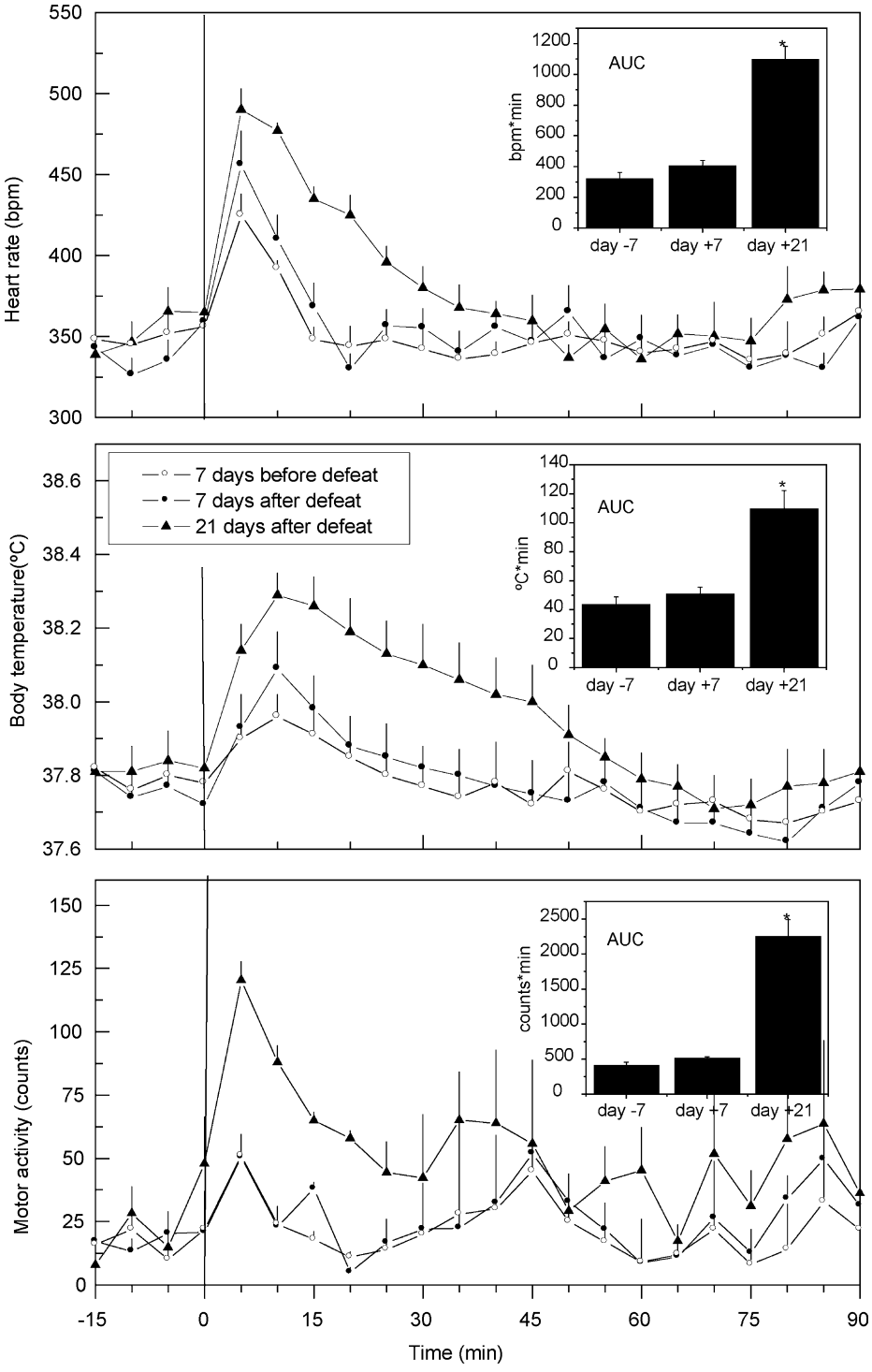

Fig. 3. Increase in body temperature, heart rate and locomotor activity (inset) in rats ($n=8$) 7 days before (pre-stress), and 7 (post7) and 21 days (post21) after a single social defeat registered in the home cage of the animals when clean bedding was offered (at $T=0$ min). A sensitization to this mild stressor was observed 3 weeks after the defeat exposure. There was a significantly higher body temperature and heart rate response in defeated rats as indicated by an increased area under the curve (AUC) measurements from $t=0$–60 min ($p<0.05$). This coincided with an increased locomotor activity after cage cleaning 21 days after defeat ($p<0.05$).

may decrease. There are a number of studies showing that the binding to hippocampal serotonergic 5-HT_{1A} receptors is decreased shortly after chronic social stress in tree shrews [57] and in subordinate rats in colony studies [58]. In a very recent study [88] it is shown that patients suffering from panic disorder have a substantially decreased binding to somatodendritic and postsynaptic 5-HT_{1A} receptors. Social defeat and psychosocial stress ('sensory contact') do not induce a change in 5-HT_{1A} receptor binding in house mice [89]. Haney et al. [90] also failed to find a lasting change in 5-HT

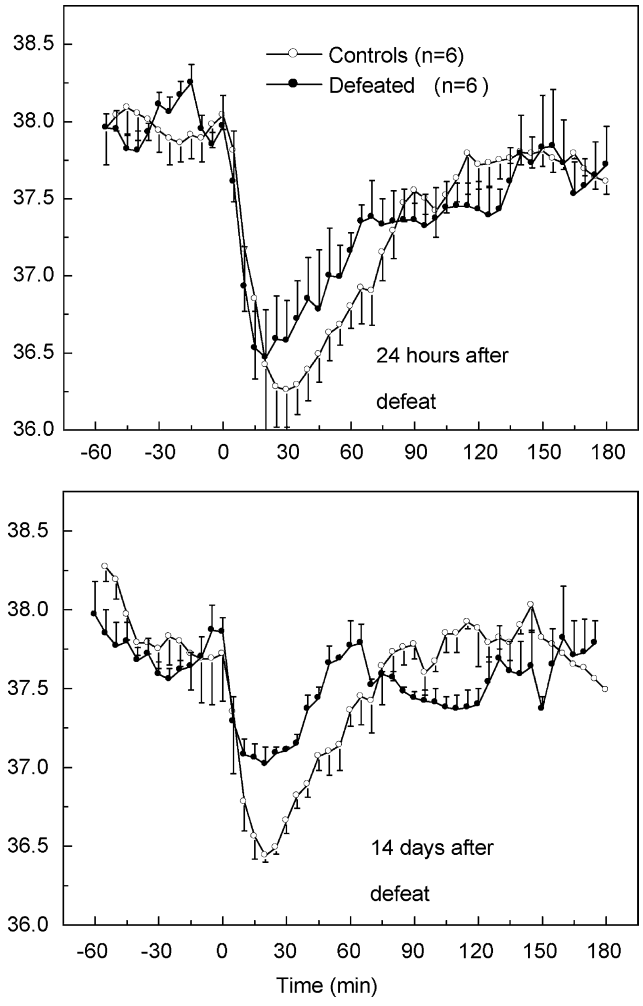

Fig. 4. Hypothermic response to the subcutaneously injected serotonergic 5-HT_{1A} receptor agonist 8-OHDPAT (0.25 mg/kg; $T=0$ min) in defeated rats 24 h before defeat and 14 days after defeat. The decrease in body temperature was compared with non-defeated controls. One day after defeat area under curve (AUC) comparisons indicate a hyposensitivity of the 5-HT_{1A} receptor that is close to significance 24 h after defeat, but progressively increases to a significant difference 14 days after defeat ($p<0.01$).

release or turnover following social defeat. Nevertheless, there are clear indications that the functionality of the 5-HT_{1A} receptor is decreased after social defeat in rats [45,91] as indicated by the physiological and neuroendocrine response to receptor agonist administration. Fig. 4 shows that, similar to the response to cage-cleaning, there is a progressive desensitization in the hypothermic response to a challenge with a 5-HT_{1A} receptor agonist, 8-OHDPAT. Since this is not caused by a decreased receptor binding, the most likely explanation is a desensitization of receptor-second messenger coupling in effector cells [92]. This desensitization of postsynaptic 5-HT_{1A} receptors in defeated rats further supports the idea that this model may be suitable to study mechanisms involved in human depression since clinically depressed patients have a desensitized 5-HT_{1A} receptor functionality [93–96].

7. A focus on structural and morphological changes in the hippocampus during and after social stress

In this review, we specifically focus on changes in hippocampal morphology and neurophysiology following social stress and how this is reflected in behavioral functions involving this structure. The impact of chronic stress on hippocampal morphology has been extensively reviewed [97–103]. These studies show how stress hormones target and modulate the hippocampus visualizing its sensitivity and plasticity to stress. Glucocorticoids in particular are the hormonal mediators of this effect of stress [99,104–107]. In social stress models like the rat social colony and the chronic psychosocial stress in tree shrews there are many indications that the hippocampus is structurally affected in the stressed animals. A volumetric reduction in the hippocampus of psychosocially stressed tree shrews was reported [108] which might be due to dendritic remodeling [56] and inhibition of neurogenesis in the dentate gyrus [109]. This impaired neurogenesis following psychosocial stress in tree shrews is mediated via excitatory amino acid input acting on NMDA receptors [109] and can be prevented by antidepressant treatment with tianeptine [108]. Tianeptine treatment also prevented the stress-induced decrease in hippocampal volume [108]. Retraction of the apical dendrites of pyramidal neurons is also reported in subordinate as well as in dominant male rats in the visible burrow [110]. Recently, we studied the effect of social defeat in rats on proliferation of cells in the dentate gyrus 24 h after the last defeat and 3 weeks after the last defeat. At both time points proliferation was reduced by more than 30% (Buwalda et al., unpublished data).

Not only morphological changes but also alterations in hippocampal receptor binding have been found after social stress. Social defeat in rats and mice decreases the ratio between mineralocorticoid and glucocorticoid receptors (MRs and GRs) in the hippocampus [50,55,89,111]. Three weeks after social defeat in rats, hippocampal MR binding was reduced to 56% as compared to non-defeated controls [55]. This finding was supported by the results of Liberzon et al. [21] and Sutanto et al. [112]. Also hippocampal serotonergic 5-HT_{1A} receptors are down-regulated in chronically psychosocially stressed tree shrews and subordinate male rats in the visible burrow [57,58].

To assess whether the changes in hippocampal morphology and receptor binding are reflected in functional aspects of this brain region, electrophysiological properties of the hippocampus were studied. Hippocampal long-term potentiation (LTP) can be evoked by brief trains of electrical stimulation that lead to a sustained increase in efficacy of synaptic transmission [113]. It is generally regarded as the primary experimental model for investigating the synaptic basis for learning and memory [114], in particular spatial learning [115,116]. This phenomenon is found to be dependent mainly upon activity of the glutamatergic NMDA receptors [117]. Von Frijtag et al. [53] and

Van der Harst [118] showed that neurophysiological aspects of hippocampal functioning as reflected in long-term potentiation (LTP) and long-term depression (LTD) are affected for extremely long time periods (up to 9 months) following defeat. These studies again indicate the importance of the housing conditions after the defeat exposure, since behavioral and electrophysiological effects of defeat could only be observed in individually housed rats [53].

In a very recent study, Kole et al. [119] compared how a brief social defeat exposure (double defeat on two subsequent days; see [50,55] and repetitive defeats (every other day for three weeks) affects functional aspects of the commissural-associational (C/A) synapses as measured by whole-cell patch-clamp recording of CA3 pyramidal neurons 22 days after the start of the defeat procedure in young adult (2.5 month old) rats. The kinetics and activity-dependent plasticity of C/A excitatory postsynaptic potentials (EPSPs) were recorded and the cells were intracellularly labeled with Neurobiotin. This marker spreads throughout the neuron including dendritic branches allowing an immunocytochemical structural analysis as also obtained in Golgi staining procedures. The C/A fibers are CA3 collaterals projecting mainly on the apical tree of neighboring CA3 pyramidal neurons [120]. The outcome of morphometric analysis of labeled CA3 pyramidal neurons in the double defeated animals and in the three weeks repeatedly stressed animals is schematically presented in Fig. 5A. Both the brief and the extended stress exposure produce dendrite retraction in the apical tree as reflected in a similar decrease in total apical dendritic length. Repeated defeat, but not double defeat, significantly reduces the total volume of the apical tree. A striking difference is observed in the effect of the two stress paradigms on the length of the basal dendrites. Whereas a double defeat induces three weeks later a strong increase in total length of basal dendrites (167% of controls) and branch complexity (number of branch 'nodes' is 176% of controls), repetitive defeat does not affect basal dendritic length as shown 24 h after the last defeat. When effects of stress on total dendritic length, apical and basal, is summed it can be concluded that shortly after repeated stress there is a clear net loss of dendrites, whereas three weeks after a double defeat, there is no loss of total dendritic length but a shift from apical to basal dendrites. This long-lasting large-scale dendritic rearrangement following a double defeat is unprecedented and leaves many questions unanswered on the temporal dynamics, the mechanisms of its regulation and of course its possible functional role.

The electrically evoked EPSPs of the C/A axons show a reduced latency of EPSP in the repeatedly defeated rats but not in double defeated animals. Furthermore, in both stress paradigms LTP could not be evoked (Fig. 5B). Only in the repetitive stress group LTP is reversed into LTD. This finding supports the findings of Von Frijtag et al. [53] who reported this phenomenon to occur up to even 9 months after the last defeat. Kole et al. [119] suggest that the slow-developing alterations of the CA3 pyramidal neurons might relate to the alterations in feedback inhibition of the HPA-axis, taking note of the strategic position of the CA3 neurons in the hippocampus and its putative trans-synaptic inhibitory connections with paraventricular neurons [121]. Although we do not know how the change in hippocampal MR/GR ratio three weeks after defeat [55] affects dendritic remodeling, it may play a role in the observed changes in

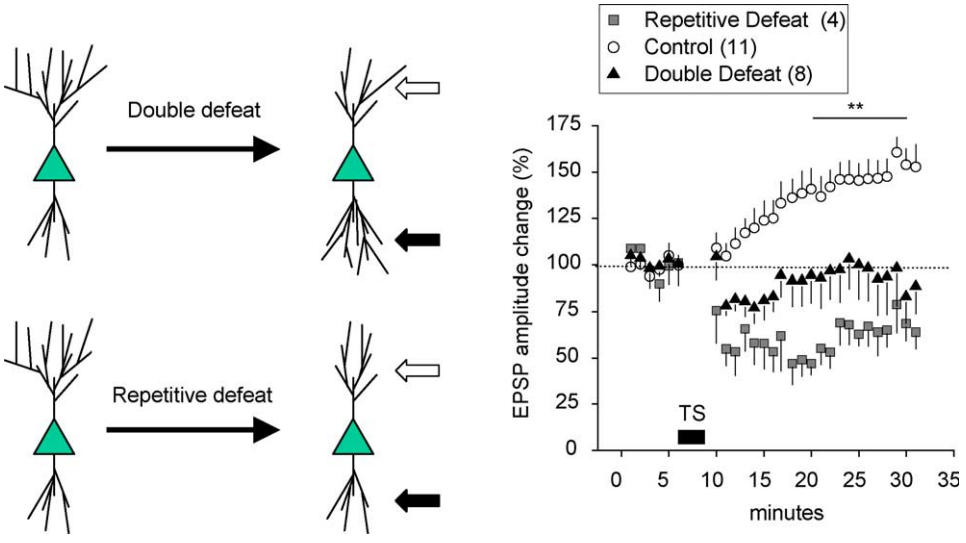

Fig. 5. Schematic representation of hippocampal CA3 pyramidal neurons 3 weeks after a double defeat on two subsequent days and 24 h after the last of a series of repetitive defeats every other day for a period of 21 days. Both after a double defeat and after repetitive defeat atrophy of the apical dendrite (white arrow) occurs. Three weeks after a double defeat experience the basal dendrites of CA3 neurons expand dramatically. This outgrowth of basal dendritic branches does not occur 24 h after the last defeat in the repetitive stress exposure. After electrical stimulation of the commissural-associational fibers, a robust LTP is induced in control animals (open circles). In double defeated rats (closed triangles) a significant inhibition of LTP is observed ($p < 0.003$). In repetitively defeated rats (gray squares) a robust LTD-like depression of excitatory postsynaptic potentials (EPSPs) is seen ($p < 0.05$).

synaptic plasticity after stress. It is known that acute injections of corticosterone produce a marked suppression in hippocampal LTP [122]. It is also known that corticosteroid receptor activation in the hippocampus produces opposite effects on LTP. Activation of MRs enhances, whereas activation of GRs suppresses LTP while at higher GR activation LTD is produced [123,124]. Therefore, at longer delays after defeat the decrease in hippocampal MR/GR ratio [50,55] possibly plays a role in hippocampal LTP suppression.

8. Functional behavioral consequences of structural and morphological changes in the hippocampus following social stress as studied in an aversive and a non-aversive learning paradigm

The hippocampal region is assumed to play a critical role in declarative memory [125–127]. It is a mediator between the initial formation of memories and their final repository elsewhere in the brain [125]. In particular it plays a role in spatial and contextual memory [128–130]. Realizing these hippocampal functions on cognitive behavior, and considering the structural and neurophysiologic changes following social defeat stress as indicated above, it is appealing to study cognitive performance in defeated rats aiming at a better insight in the functional behavioral consequences of social stress involving this brain structure.

Surprisingly, the contextual memory performance of defeated rats 35 days after the stress was not impaired but, as mentioned, it is possible that this test is not the most favorable in dissociating differences in fine-tuning of hippocampal functioning. We, therefore, measured spatial learning and memory skills in rats three weeks after a prior defeat. Rats were confronted with a resident male on five subsequent days until a submissive posture was assumed (within 2 min after introduction in resident's cage). The stress period was prolonged for up to 1 h by placing the intruder rats in their own home cage in the larger cage of the resident allowing auditory, visual and olfactory contact inducing psychosocial stress without further physical contact [44]. Rats were subsequently tested in the Morris water maze starting 24 days after the last defeat and their performance was compared with non-defeated controls. In the Morris water maze rats have learn to find a hidden escape platform [131,132]. Escape latency and distance swum were used as measures for behavioral performance during the learning phase. The animals were offered two learning trials per day for four days with an inter-trial time of 1 h. On the fifth day the platform was removed (in the so-called probe trial) in order to see whether the rats have a spatial preference for the former platform location. The learning performance of defeated rats was not impaired as indicated by the escape latencies (Fig. 6). Actually the learning in defeated rats is superior to non-defeated controls ($p<0.05$). There was no significant difference in spatial

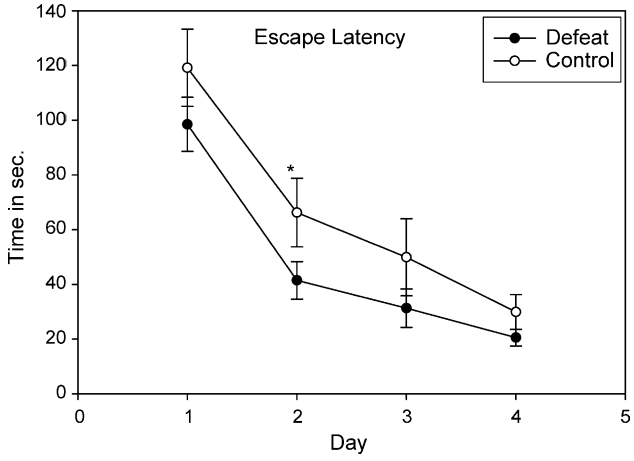

Fig. 6. Escape latencies to hidden platform in the Morris water maze learning paradigm that started 3.5 weeks after the last defeat on four training days in defeated rats ($n=17$) and non-defeated controls ($n=15$). Each time point is the result of taking the mean value of two trials, with an intertrial time of 1 h. Defeated rats show on average an increased learning behavior as indicated by decreased escape latency ($p<0.05$). Socially stressed rats were confronted five times on subsequent days with a resident male.

preference reflecting reference memory as measured in the probe trial. Both groups spend significantly more time in quadrant 1 in which the platform was placed during the learning trials.

Next to the aversively motivated Morris water maze, cognitive performance was also measured in a reward motivated learning task. In the food-rewarded hole-board learning paradigm [133–135] rats have to find four baited holes out of a total of 16 holes. Defeated and non-defeated rats were trained for 8 days in this learning task. On these 8 days rats were offered two trials per day with an inter-trial time of 1 h during which they were allowed to find the four rewards (which were small pieces of cheese). The first trial started 22 days after the last defeat. During the hole-board exploration, behavior of the rats was video observed and all visits and re-visits to baited and non-baited holes were quantified. From these observations, working (no. food rewarded visits/no. visits + no. re-visits to baited holes) and reference memory (no. visits + no. re-visits to baited holes/total no. of visits) were calculated. Defeated rats did not show a difference in reference memory (Fig. 7) nor in working memory (data not shown).

The results from these two learning and memory tasks indicate that learning and memory performance in defeated rats is not impaired 3 weeks after defeat. The results in the water maze learning show that learning might be even improved in previously socially stressed rats. This may seem surprising since there is a massive amount of literature on stress and cognition indicating that cognitive functioning is impaired after stress [136–144].

The long-lasting effect of defeat on structural changes in the hippocampus, i.e. the shortening of apical dendrites of CA3 pyramidal neurons [119] and on its electrophysiologically

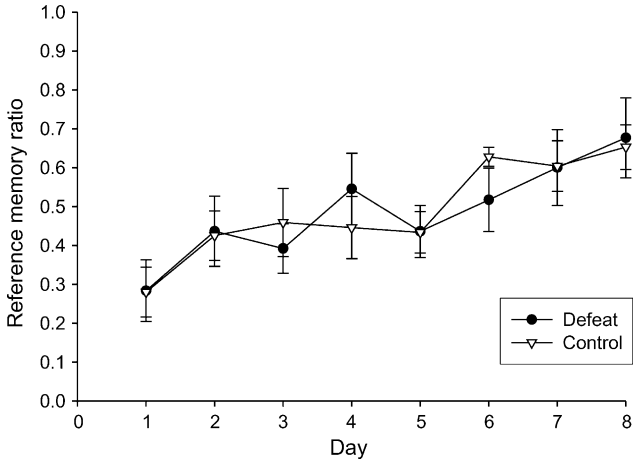

Fig. 7. Reference memory performance as measured in the hole-board learning paradigm that started 22 days after the last defeat in rats that have been confronted five times with a resident male conspecific. Behavioral performance was compared with non-defeated controls. There was no significant difference in reference memory performance between stressed and non-stressed rats.

registered decreased LTP [53,119] intuitively supports a decreased cognitive functioning. However, almost all previously described negative effects of stress on cognitive performance were studied in animals immediately or shortly after the end of a chronic stress period or an acute stressor. At that moment, neuroendocrine activation resulting in elevated circulating corticosterone levels may play an important role in cognitive effects of stress since the crucial role of an increased corticosteroid secretion on this behavior is generally acknowledged [105,107,145].

A study in tree shrews [140] has measured hole board learning both during and at different time intervals after a psychosocial stress episode. In that approach the main negative effect of stress on spatial performance was found in the testing sessions that were performed at delay intervals of 1 week and 10 weeks after a stress episode while during the stress spatial performance was only modestly affected. In that study urinary cortisol secretion levels were elevated for the whole stress period of 8 weeks. However, at the impaired performance 10 weeks after the last stress exposure cortisol secretion was back to pre-stress levels.

A later study in the same animal species shows that during chronical psychosocial stress, tree shrews have a significantly enhanced spatial learning in the same spatial learning task, being the food rewarded hole-board learning paradigm [146]. This coincided with a decreased neurogenesis in the dentate gyrus granular cell layer. It is also known from other studies applying this animal model of social stress that in CA3 neurons the apical dendrites retract [56]. Other behavioral cognitive studies during or shortly after the cessation of stress also show that the effect of stress on learning and memory performance is not always negative [86,147]. In these studies the facilitation of contextual fear conditioning involving hippocampal functioning was studied. This facilitation was suggested to be mediated by a disinhibition of the HPA-axis [86,147]. We cannot explain the differences between the two studies in psychosocially stressed tree shrews. Neither can we explain why rats show an unaffected (hole-board learning) or an improved (Morris water maze) cognitive performance three weeks after the end of a social stress experience, whereas tree shrews appear to be performing superior or similar during psychosocial stress but show impaired performance at longer time intervals. Since many of these animal models are aimed at mimicking stress-related psychopathology it is important to realize that studies in outpatient depressed young adults do not show a major negative effect on cognition [148].

The structural changes in the hippocampal formation following stress and the impact of stress on the electrophysiological properties of this structure also have been addressed mainly in studies at the end of an acute or chronic stress period [56,141,144,149]. The findings in these acute or chronic stress studies have led many researchers to the suggestion that hippocampal LTP may be the neural basis for hippocampus dependent learning. Whether this suggestion is valid remains to be proven in a conclusive way [150], since it has been extensively shown that spatial learning is possible without NMDA dependent hippocampal LTP [151–154]. The present behavioral cognitive findings in combination with the electrophysiological data of Kole et al. [119] confirm that an absent LTP not necessarily results in impaired spatial learning performance. The differential effect of repetitive social defeat stress *without*, and brief stress *with* a time delay on dendritic remodeling in hippocampal CA3 neurons may be an additional factor in the temporal dynamics that occur in the interaction between stress and cognitive performance. In particular the shift from apical dendritic branches to basal dendritic outgrowth [119] may play an intriguing role in this that needs further study.

9. Conclusion

Social defeat stress can affect behavior, physiology, neuroendocrinology and brain for a long period of time after the end of the stress exposure. There are a number of experimental conditions, like resisting defeat during, and housing conditions after the conflict that play an important role in the magnitude and duration of the stress effect. Counter-fighting while being attacked by the resident reduces the stress effects [46]. Housing conditions play a crucial role in the effects of defeat since they are only observed in individually housed rats [47–49].

Where structural and electrophysiological properties of the hippocampus show to be highly sensitive to the long-term effects of defeat, this stressor fails to induce a lasting impairment in cognitive behavioral functioning in rats. These seemingly contradictory findings may tell us a number of things. The first one is that our behavioral tools

to measure cognitive functioning in experimental animal research might not be sensitive enough to monitor the functional consequences of the stress-induced changes in the hippocampal formation. The second one is that changes in hippocampal morphology and neurophysiology as observed after stress are not by definition indicators of stress-related psychopathology. The findings show that cognitive performance involving this structure is not impaired even though structural and electrophysiological changes are maintained for long time periods after the end of the stress exposure. This supports the idea that the brain, and specifically the hippocampus, uses its large neuronal plasticity for a successful adaptation to the stressful situation that occurred previously. A major factor in this adaptive capacity may be that the plasticity observed in the hippocampus is an important, but not complete picture of what is going on in the brain after stress. It is likely that changes that occur in the hippocampus are accompanied by changes in other brain regions functionally related to the hippocampus. These changes throughout the brain reflect a process where reacting and changing neurotransmitter systems and brain areas are interacting in a concerted fashion aimed at maintaining homeostasis. This process of allostasis or 'maintaining stability through change' in physiological coping mechanisms has been extensively described before [155–157].

The differences that exist in the outcome of studies applying chronic stress and studies addressing long-lasting changes of brief stressors are probably less related to the intensity or duration of the applied stress than to the temporal dynamics of the changes observed. Both immediately after chronic stress as well as long time after brief social stress, hippocampal morphology is affected. However, in these two experimental approaches the behavioral functionality involving these structural changes can be very different. At shorter time intervals after stress, the changes in various brain regions may not yet be in balance due to regional differences in the temporal dynamics in the process of neuronal adaptive plasticity. At longer time delays after the stress behavioral and functional recovery sets in even though structural and electrophysiological traces or 'scars' remain visible at a local level. Whether or how these neurobiological traces of previous stress experiences contribute to an altered vulnerability to subsequent stressful events, remains to be addressed in further research.

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